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Isolation of Stigmasterol from Alcoholic Extract of Bark of Calotropis Procera

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ABSTRACT

Aim of this work is to isolate Stigmasterol from the dried bark powder of Calotropis procera by applying hot extraction and then the crude methanol extract was fractionated with ethyl acetate, petroleum ether and chloroform.

Keywords: Stigmasterol, Calotropis procera.

INTRODUCTION

Research works including extraction, isolation and identification have now formed the major field of study. The plant Calotropis procera locally known as Aak belongs to the Asclepiadaceae family[1] and it is available abundantly on malva plateau as well as throughout India. Its study is important because of its Energy and Chemicals.The whole plant parts (leaf, stem, bark) were known to have oil, polyphenol, hydrocarbons, lignin, crude protein, α-cellulose and ash[2]. The bark powder of the plant is known to have highest amount of hydrocarbons whereas leaves have the lowest amount. This plant is having highest gross heat value suitable as alternative source of hydrocarbons and others phytochemicals, out of which attempt has been made to isolate Stigmasterol using the dried bark powder of Calotropis procera. Although Calotropis procera also has anticonvulsant, analgesic, anxiolytic and sedative effect (Pathak 2006). But all these studies are not sufficient for the bioactive compounds characterization and identification.

MATERIALS AND METHOD

Plant Material Extraction

The plant parts of Calotropis procera were collected during the month of May-June, 2013 from the study area (Mhow) of Govt. P. G. College Campus.The plant was taxonomically identified by professor Dr. H. N. Satya, retired, Department of Botany, Govt. Autonomous Holkar Science College,

Indore and a specimen was kept in the Deptt. on dated 21.05.2013.

The dried bark powder (1.6 kg) was subjected under soxhlet extractor for the hot extraction with methyl alcohol. After this process material is evaporated and 160 g crude extract was collected.Twenty five grams of the crude methyl alcohol extract was fractionated into chloroform fraction (2g), petroleum ether fraction (15 g), ethyl acetate fraction (2 g) (Bahl and Bahl, 1992)[3].

Isolation of Compounds

A portion of petroleum ether fraction of both cold and hot extraction was dissolved in petroleum ether and this solution was spotted on TLC plate. These plates were run by specific solvent system and were viewed under UV light (Bobblt, 1963)[4] and vanillin H_2SO_4 reagent. These compounds were separated by use of solvent system of n-hexane and ethyl acetate in the proportion of 9:1.The ten grams of the petroleum ether was subjected to column chromatography on the silica gel[5](40-100) with gradient elution using n-hexane, ethyl acetate and finally with 100% methanol (Shrivastave, 1987)[6]. A fraction was found homogenous on TLC plate by using petroleum ether: ethyl acetate (9:1), n-hexane: chloroform(10:1) and petroleum ether: methanol (7:3) solvent systems. These fractions were dcrystallized and isolated as CGI-01.

Liebermann-Burchard reaction

A few crystal of isolated CGI-01 were dissolved in chloroform and a few drops of concentrated sulphuric acid was added to it followed by the addition of 2-3 drops of acetic anhydride solution. It get turned into violet, blue and then finally to green[7].

Test for Steroid

A few crystals were dissolved in chloroform and a few drops of concentrated sulphuric acid was added then a reddish colour was seen in the upper layer of chloroform solvent[8].

Test for Alcohol

Cerric ammonium nitrate reagent was prepared by dissolving 10 mL^{-1} of 2N HNO_3 on mild heating. A few crystal of isolated material were dissolved in 0.5 mL^{-1} of dioxane.The solution was mixed with 0.5mL^{-1} with dioxane to get yellow to red colour which confirms the presence of an alcoholic hydroxyl group[9].

RESULT AND DISCUSSIONS

The melting point of CGI-01 was found to be 176˚C, gave positive test of alcohol and steroids .Thus it is assumed to be a sterol. The melting point of CGI-01 was in fine agreement with the melting point given for Stigmasterol in the literature.

CONCLUSION

From the available and confirmed physical and chemical evidence as obtained from the experiments and literature CGI-01 was confirmed as available standard structure of Stigmasterol.

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